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PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

BECKER, Konrad
Novartis AG
Patent- und Markenabteilung
Lichtstrasse 35
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SUISSE

Date of mailing (day/month/year)
08 November 1999 (08.11.99)

Applicant's or agent's file reference
PH/5 -30021/A

IMPORTANT NOTIFICATION

International application No.
PCT/EP98/03279

International filing date (day/month/year)
02 June 1998 (02.06.98)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address
BOUTSALIS, Peter
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CH-4059 Basel
Switzerland

State of Nationality: AU
State of Residence: CH
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
BOUTSALIS, Peter
Känelmattstrasse 37
CH-4422 Arisdorf
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State of Nationality: AU
State of Residence: CH
Telephone No.:
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Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

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F. Gateau

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PATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
PH/5 -30021/A		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 98/ 03279	02/06/1998	04/06/1997
Applicant		
NOVARTIS AG et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [ ] the text is approved as submitted by the applicant
   - [X] the text has been established by this Authority to read as follows:

   PESTICIDE SCREENING SYSTEM

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. -
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PH/5 -30021/A		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP98/03279	02/06/1998	04/06/1997
International Patent Classification (IPC) or national classification and IPC G01N33/50		
Applicant NOVARTIS AG et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which hav been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17/11/1998	19.03.99
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. (+49-89) 2399-0 Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer Wieser, M Telephone No. (+49-89) 2399 8434

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP98/03279

I. Basis of the r port

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

15	as received on	10/03/1999	with letter of	09/03/1999
1-14,16-26	as originally filed			

Claims, No.:

1-21	as received on	10/03/1999	with letter of	09/03/1999

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-21
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-21
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-21
	No:	Claims	

2. Citations and explanations

see separate sheet

Section V:

Claims 1-21 refer to methods for testing asexually propagated plants within a plant screening program, wherein the propagation step is accomplished without passing through a callus phase or involving cell or protoplast culture.

In the light of the disclosure in the documents cited in the International Search Report, novelty and inventive step (Articles 33(2) and 33(3) PCT) of claims 1-21 is acknowledged.

What we claim is:

1. Test system for testing progeny plants, comprising

(a) asexually propagating progeny plant(s) from a mother plant without passing through a callus phase or involving cell or protoplast culture.;

(b) incorporating the so obtained progeny plant into a plant screening program; and

(c) monitoring the growth of the progeny plant.

2. Test system according to claim 1, wherein the propagation step is accomplished by

(a) cutting a short segment from a mother plant such that said segment is capable of directly regenerating into a whole and morphologically normal plant,

(b) transferring said excised segment to a suitable anchorage material; and

(c) regenerating said transferred segment into a whole and morphologically normal plan without passing through a callus phase or involving cell or protoplast culture.t

3. Test system according to claim 2, wherein said segment comprises a region that contains a high amount of actively dividing cells.

4. Test system according to claim 3, wherein said region comprises meristematic cells.

5. Test system according to claims 3 and 4, wherein said segment comprises a short root and shoot fragment.

6. Test system according to claim 2, wherein the anchorage material is

(a) an inert material such as vermiculite, perlite or plastic beads;

(b) a culture medium commonly applied in plant cultivation; or

(c) soil.

Replaced by Article 34

7. Test system according to any of the previous claims wherein said test system is used within a high through-put format.

8. Test system according to claim 1, comprising

(a) cutting a short segment from a mother plant such that said segment is capable of directly regenerating into a whole and morphologically normal plant,

$(b)_1$ dipping said segment into a known concentration(s) of a pesticide-containing solution; or, in the alternative

$(b)_2$ spraying said segment with a known concentration(s) of a pesticide-containing solution;

(c) transferring the so treated plant explants to a suitable anchorage material;

(d) regenerating said explant into a whole and morphologically normal plant without passing through a callus phase or involving cell or protoplast culture.; and

(e) monitoring the growth of the progeny plant.

9. Method of rescuing plants showing an interesting trait or property after treatment with a pesticide for further investigation comprising

(a) asexually propagating progeny plant(s) from a treated mother plant without passing through a callus phase or involving cell or protoplast culture.;

(b) incorporating the so obtained progeny plant into a plant screening program; and

(c) monitoring the growth of the progeny plant.

10. Method according to claim 9, wherein the propagation step is accomplished by

(a) cutting a short segment from a treated mother plant such that said segment is capable of directly regenerating into a whole and morphologically normal plant,

(b) transferring said excised segment to a suitable anchorage material; and

(c) regenerating said transferred segment into a whole and morphologically normal plant without passing through a callus phase or involving cell or protoplast culture..

11. Method according to claim 9 comprising

(a) cutting a short segment from a treated mother plant such that said segment is capable of directly regenerating into a whole and morphologically normal plant,

$(b)_1$ dipping said segment into a known concentration(s) of a pesticide-containing solution; or, in the alternative

$(b)_2$ spraying said segment with a known concentration(s) of a pesticide-containing solution;

(c) transferring the so treated plant explants to a suitable anchorage material;

(d) regenerating said explant into a whole and morphologically normal plant without passing through a callus phase or involving cell or protoplast culture.; and

(e) monitoring the growth of the progeny plant.

12. Method according to any of the previous claims, wherein the pesticide is selected from the group consisting of a herbicide, an insecticide and a fungicide.

13. Method for determining whether a resistance phenotype observed in a plant is due to a resistance trait or caused by other factors, comprising

(a) collecting the phenotypically resistant plant

(b) asexually propagating progeny plant(s) from said plant without passing through a callus phase or involving cell or protoplast culture.;

(c) incorporating the so obtained progeny plant into a plant screening program; and

(d) monitoring the growth of the progeny plant.

14. Method according to claim 13, comprising

(a) collecting the phenotypically resistant plant

(b) cutting a short segment from said plant such that said segment is capable of directly regenerating into a whole and morphologically normal plant,

$(c)_1$ dipping said segment into a known concentration(s) of a pesticide-containing solution; or, in the alternative

$(c)_2$ spraying said segment with a known concentration(s) of a pesticide-containing solution;

(d) transferring the so treated plant explants to a suitable anchorage material;

(e) regenerating said explant into a whole and morphologically normal plant without passing through a callus phase or involving cell or protoplast culture.; and

(f) monitoring the growth of the progeny plant.

15. Method according to claims 13 and 14, wherein the resistance phenotype is observed after treating the plant with a pesticide.

16. Method according to claim 15, wherein the pesticide is selected from the group consisting of a herbicide, an insecticide and a fungicide.

17. Method according to any of the preceding claims, wherein the plant to be tested is a weed plant.

18. Method according to any of the preceding claims, wherein the plant to be tested is a crop plant.

19. Method according to any of the preceding claims, wherein the plant to be tested is a transgenic plant.

20. Use of a test system according to any one of claims 1-8 for rescuing plants showing an interesting trait or property after treatment with a pesticide for further investigation.

21. Use of a test system according to any one of claims 1-8 for determining whether a resistance phenotype observed in a plant is due to a resistance trait or caused by other factors.

22. Test-kit in a ready to use format comprising all devices necessary to carry out the test system according to any one of claims 1-8.

23. Test kit in a ready to use format according to claim 22 comprising

(a) a cutting device, preferably a knife or sharp scissors to cut the weeds out of the ground, bags to put them in, pots, test tubes containing an anchorage material, preferably agar, soil, sand, vermiculite or a mixture of one or more of said components, etc, to grow them in, pesticide containing solution to spray with, a spraying device and an instruction manual;

(b) a cutting device, preferably a knife or sharp scissors to cut the weeds out of the ground, bags to put them in, pots, test tubes containing an an anchorage material, preferably agar, soil, sand, vermiculite or a mixture of one or more of said components, etc, to grow them in, one or more container containing pesticide solution(s) with different concentrations of pesticide for dipping the plant cuttings into a known concentration(s) of pesticide and an instruction manual;

(c) a cutting device, preferably a knife or sharp scissors to cut the weeds out of the ground, bags to put them in, pots, one or more container containing an appropriate anchorage material such as, for example, agar, soil, sand, vermiculite or a mixture of one or more of said components, wherein said anchorage material already contains uniformly distributed therein a pesticide solution with different concentrations of pesticide, and an instruction manual.

24. A test kit according to any of the previous claims, wherein the pesticide is selected from the group consisting of a herbicide, an insecticide and a fungicide.

phenotype can be rescued and further investigated thereby confirming that the individual plant or plants are truly resistant.

Structural genes which are preferably used to confer a particular resistance trait to a plant are those which code for proteins which are able to protect plants against pathogens (for example phytopathogenic fungi, bacteria, viruses etc.), herbicides (for example triazines, sulfonylureas, imidazolinones, triazolepyrimidines, bialaphos, glyophosates, etc.), fungicides, insecticides or disadvantageous environmental influences (for example heat, cold, wind, unfavorable soil conditions, moisture, dryness, etc.).

Within the scope of this invention, the use of transformed plants comprising structural genes associated with the control of plant pathogens and parasites are particularly preferred.

For example, resistance towards insects can be transferred by a gene which codes for a polypeptide which is toxic for insects and/or their larvae, for example the crystalline protein of *Bacillus thuringiensis*. Such genes are known and described, for example, in US-P 4,865,981, US-P 4,996,155, WO 89/07605, EP 213,318 and EP 186,379. A further class of vegetative insecticidal protein encoding DNA sequences is described in WO 94/21795 and WO 96/10083.

The protease inhibitors are a second class of proteins which mediate insect resistance. Protease inhibitors form a normal constituent of plant storage structures and for this reason are usually located in vacuoles or "protein bodies". Thus, it was possible to demonstrate that the Bowman-Birk protease inhibitor, which was isolated and purified from soya beans, inhibits the intestinal protease of Tenebrio larvae (Birk et al., 1963). The gene, which codes for a trypsin inhibitor from the common cowpea, is described in Hilder et al. (1987).

Within the scope of the present invention, plants comprising any *Bacillus thuringiensis* crystal protein, vegetative protein, protease inhibitor or any other insecticidal protein-encoding DNA sequences can be used within the testing system according to the invention, irrespective of their provenance (e.g. insecticidal proteins from non-plant sources or from purely synthetic sources).

In this connection, mention must also be made of hydrolytic enzymes which are either able on their own to bring about degradation of the cell walls of plant pathogens or else